IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Remacle et al.
Appl. No.	:	10/056,229
Filed	:	January 23, 2002
For	:	IDENTIFICATION OF A LARGE NUMBER OF BIOLOGICAL (MICRO)ORGANISMS GROUPS AT DIFFERENT LEVELS BY THEIR DETECTION ON A SAME ARRAY
Examiner	:	Woolwine, Samuel C.
Group Art Unit	:	1637

DECLARATION UNDER 37 C.F.R §1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. This Declaration is being submitted to demonstrate that the claimed invention unexpectedly provides the ability to rapidly determine which of several nucleic acids having greater than 30% homology with one another is present in a sample with high sensitivity and specificity.

2. I am an inventor on the above-identified patent application and am familiar with the specification and prosecution history.

3. I have extensive experience in the field of the claimed invention as indicated in the attached Curriculum Vitae provided herewith as Exhibit A.

4. The claimed invention provides accurate discrimination between homologous sequences and yields results within 60 and even within 30 minutes of the conclusion of the hybridization step. In order to provide specificity, the claimed invention employs a capture molecule comprising a short sequence which is between about 15 and about 40 bases in length and which is specific for the target sequence. In order to provide specific features related to the invention as

explained below, the sequence specific for the target sequence is linked to a nucleic acid spacer which is at least 40 nucleotides in length.

5. The present invention was designed to overcome a problem in prior art methods for identifying and/or quantifying the biological organisms. The problem with identifying homologous sequences on an array is that one has to have good sensitivity to be able to detect the signal combined with good specificity to be able to distinguish among different but homologous sequences present in a sample. The process of identifying should not be overly time consuming. The problem in the prior art was that in order to obtain good specificity of detection of long target molecules, short capture probes attached to a solid support without a spacer could be employed. However, such a method would result in the detected signal being too low, i.e. the sensitivity of the method will be too low. Moreover, the kinetics of the reaction are poor, because the two strands of the long target sequence will preferentially re-anneal to one another rather than hybridizing to short capture nucleotide sequences immobilized on a surface, resulting in low sensitivity. On the other hand, if the capture probe is long, then the method of detection of long target probes is sensitive, but has low specificity due to non-specific cross-reaction of the capture probe with the other species of the target molecules present in a sample.

6. To avoid the kinetic problem, prior art assays have typically used short capture sequences for short targets or long capture sequences for long targets. However, either option necessarily invokes one of the disadvantages discussed above.

7. The inventors unexpectedly found that if a single-stranded capture molecule has a nucleic acid spacer of at least 40 bases long connected to the surface of the solid support, then the detection of long target sequences (100-800 bp) using short specific capture probes gains in sensitivity without losing high specificity, AND the detection of long target sequences using overall long capture probes gains in specificity without losing high sensitivity. Keeping the detection sensitivity together with the specificity for detection of homologous amplicons was an unexpected result of the present invention.

8. The ability of the claimed invention to accurately discriminate between homologous sequences and to rapidly yield results is extremely valuable in commercial applications. The product to be used with the methods of the current invention is expected to be put on the market soon to achieve fast and safe analysis of genetically modified organisms in foodstuff. The

product to be commercialized is referred to as the Eppendorf DualChip® GMO microarray (see Exhibit 1).

9. In order to provide high sensitivity, the claimed invention utilizes a relatively long double-stranded target sequence which is between 100 and 800 bases in length. The use of such long sequences allows to increase the sensitivity of the methods since many labels can be incorporated into a such a long DNA fragment during the amplification step. Also, there is no need to cut the amplicons into fragments for the setection which makes the method of the invention easy to use since the PCR solution can be directly hybridized on the array without any further treatment. However, such long target sequences do not hybridize as efficiently to short capture sequences as to long capture nucleotide sequences. The present invention overcomes this problem by linking the short capture nucleotide sequence to a nucleic acid spacer which is at least 40 nucleotides in length.

10. Using a longer capture nucleotide sequence would enable the capture nucleotide sequence to more efficiently hybridize to long target sequences. However, use of long capture nucleotide sequences results in reduced specificity. The present invention overcomes this problem by linking a short capture nucleotide sequence which is about 15 to about 40 nucleotides in length to a nucleic acid spacer which is at least 40 nucleotides in length.

11. Exhibits 2-6 show the data obtained by the inventors in support of the advantages of the claimed method.

12. Exhibit 2 shows that hybridization of long double-stranded target molecules to short capture probes which are not linked to a spacer is specific, but the obtained signal is too low. In the experiment of Exhibit 2, double-stranded target molecules of 489 bp of the *S. epidermidis FemA gene* were hybridized to short capture probes from the *FemA* genes from *S. aureus, S. epidermidis, S. haemolyticus, S. hominis,* and *S. saprophyticus* which were 27 bases in length and which lacked a nucleotide spacer. The protocol provided in Example 2 of the present application was used to conduct the analysis. As depicted in Exhibit 2, a signal is only observed on the *S. epidermidis* capture probe but the signal value is very low (signal value = 13) and almost undetectable. Thus, use of a short capture probe which lacks a spacer provides specificity but not sensitivity.

13. Exhibit 3 shows that hybridization of long double-stranded target molecules to long capture probes without a spacer gives a good signal but is non-specific. Double-stranded target molecules of 101 bp of the *S. epidermidis FemA* gene were hybridized to long capture probes of 101 bases for *S. epidermidis*, 108 bases for *S. saprophyticus*, 139 bases for *S. aureus*, 128 bases for *S. haemolyticus* and 118 bases for *S. hominis*. The protocol of Example 1 of the present application was followed in this experiment. As depicted in Exhibit 3, the target *S. epidermidis* amplicons were able to specifically bind to the capture probe for *S. epidermidis* with a high signal value (152), but they also non-specifically bound to the other 4 capture probes (signal value = 144, 9, 13 and 20 respectively for the *S. saprophyticus*, *S. aureus*, *S. haemolyticus* and *S. hominis* capture nucleotide sequences). In conclusion, the use of long capture sequences with long target sequences provides sensitivity but not specificity.

14 Exhibit 4 shows that when a short capture probe includes a nucleic acid spacer, it hybridizes with its target with high specificity and also gives a good signal. In fact, the signal level is similar to that obtained with a long capture probe that does not include a nucleic acid spacer. In the experiment of Exhibit 4, a double stranded target molecule of 587 bp from the *S. aureus FemA* gene was hybridized to long capture probes comprising of a spacer of 40 nucleotides and a short sequence of 27 nucleotides specific for the *FemA* genes of different *Staphylococci* species. The protocol of Examples 4 and 7 of the present application were utilized for this experiment and the resulting signals were quantitated. A consensus capture probe of 489 nucleotides which hybridizes to the *FemA* gene of all of the foregoing species of *Staphylococcus* was included as a positive control. As depicted in Exhibit 4, a signal was only observed on the capture probe specific for *S. aureus* and on the consensus capture probe.

15. In theory, the rate of hybridization is proportional to the square root of the smaller sequence taking part to the hybridization (i.e. the sequence of the capture probe of 489 or 27 bases). Accordingly, the reassociation of long amplicons of 587 bases or the hybridization of the amplicon on the capture probe of 489 bases should be much faster than the hybridization of the amplicon on a capture probe of 27 bases. However, uunexpectedly, the signal value obtained with the *S. aureus* specific capture probe comprising 27 nucleotides complementary to the target was the same as that obtained with the 489 nucleotide consensus probe the signal values were very high (signal values = 152 and 158 respectively for *S. aureus* and the consensus capture

nucleotide sequences). The signal values obtained with the 27 nucleotide capture probe linked to the spacer demonstrates the high sensitivity of the claimed invention.

16. Exhibit 5 shows that even when a plurality of amplified targets is present in a sample, the use of a short specific capture probe linked to a spacer of at least 40 nucleotides, allows obtaining specific signals of high values (i.e. the method is sensitive and specific) Double stranded targets of 587 bp of the *S. gallinarum FemA* gene and the *S. saprophyticus FemA* gene were generated by amplifying the two targets using consensus primers as described in the specification of the present application. The amplified targets were hybridized to capture probes comprising a spacer of 40 nucleotides and a short (27 bases in length) sequence specific for the target sequences using the protocols of Examples 4 and 7 of the present application and the resulting signals were quantitated. A signal was only observed on the specific capture probes of *S. gallinarum* and *S. saprophyticus* and the signal values were very high (signal value = 169, 136 and 167 respectively for *S. gallinarum*, *S. saprophyticus* and the consensus capture nucleotide sequences). There is an unambiguous detection of the two bacterial species present in the sample. In conclusion, the assay is both sensitive and specific even when a plurality of targets is present in the sample.

17. Exhibit 6 shows that the length of the spacer is important for the sensitivity of detection of the hybridization of a long double-stranded target on short specific capture molecules linked to a nucleic acid spacer. A long double stranded target of 587 bp of the *S. epidermidis FemA* gene was hybridized to capture probes having spacers of different lengths (20, 40, 85 and 95 bases) linked to a sequence specific for the target which was 27 bases in length. As shown in Exhibit 6, the rate of hybridization was increased by a factor of 5 for a spacer portion of 95 nucleotides, as compared to a spacer portion of 20 nucleotides when keeping constant the sequence of the capture probe specific for the amplified target.

18. This is important in the context of a microarray because the hybridization conditions (temperature, buffer) for all of targets to be assessed on the array will be uniform while the optimal hybridization temperature for a given target could be different from another target. Enhancing the signal levels using spacers of the lengths recited in the claims enhances the ability to evaluate the binding of multiple targets under uniform conditions.

19. The composition of the spacer is also very important and contributes to the unexpectedly high sensitivity observed. A spacer which is a nucleotide sequence gives a higher hybridization

signal than a non-nucleotide spacer. The scientific explanation for such sensitivity improvement using as spacer a nucleotide sequence is not clear. One hypothesis is that the spacer plays a role not only in reducing the steric hindrance of the reaction near the surface but that the presence of non-specific DNA bases in the spacer plays an important role in increasing the extent of hybridization. This could result from non-specific interactions which are high enough to attract the target sequences to the capture probes but not so high as to result in non-specific binding under the experimental conditions are used. This hypothesis for the exceptionally good hybridization results is derived from the observation by the inventors that not all the DNA sequences are equally efficient as spacers.

20. The ability of nucleotide spacers at least 40 nucleotides in length to dramatically improve the extent of hybridization and the foregoing hypothesis were not known nor suggested at the time this application was filed.

21. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 14 July 2006

By: _____
Jose Remacle

2711494
062606

Eppendorf DualChip® GMO microarray for fast and safe analysis of genetically modified organisms in foodstuff

The new EU-wide regulations for gene technology identification define that more than 0.9% GMO (genetic modified organism) in foodstuff must be identified. Detection of these recognized GMOs is challenging in itself, and many new GMOs are currently in development or are waiting for their EU approval.

The DualChip GMO kit, based on Eppendorf's unique and innovative microarray technology, easily and conveniently enables the detection of GMOs in food in a highly standardized manner. As new GMOs are developed and approved for use, they can also be quickly and easily added to the existing microarray. For even quicker results, DualChip GMO can be used with the Eppendorf Silverquant® detection system to automatically analyze all GMO markers in parallel with high sensitivity and process safety.

Due to the microarray format, newly approved GMOs can easily be added. Eppendorf will initiate the technological validation by the EU within quarter 2 2006.

Article By

Eppendorf AG
Jens Behrens
Barkhausenweg 1
22339 Hamburg
Deutschland
T: ++ 49 40 53 8010
F: ++ 49 40 53 801-556

- **Supplier details**
- **Send email**
- **Supplier website**
- **Supplier news archive**

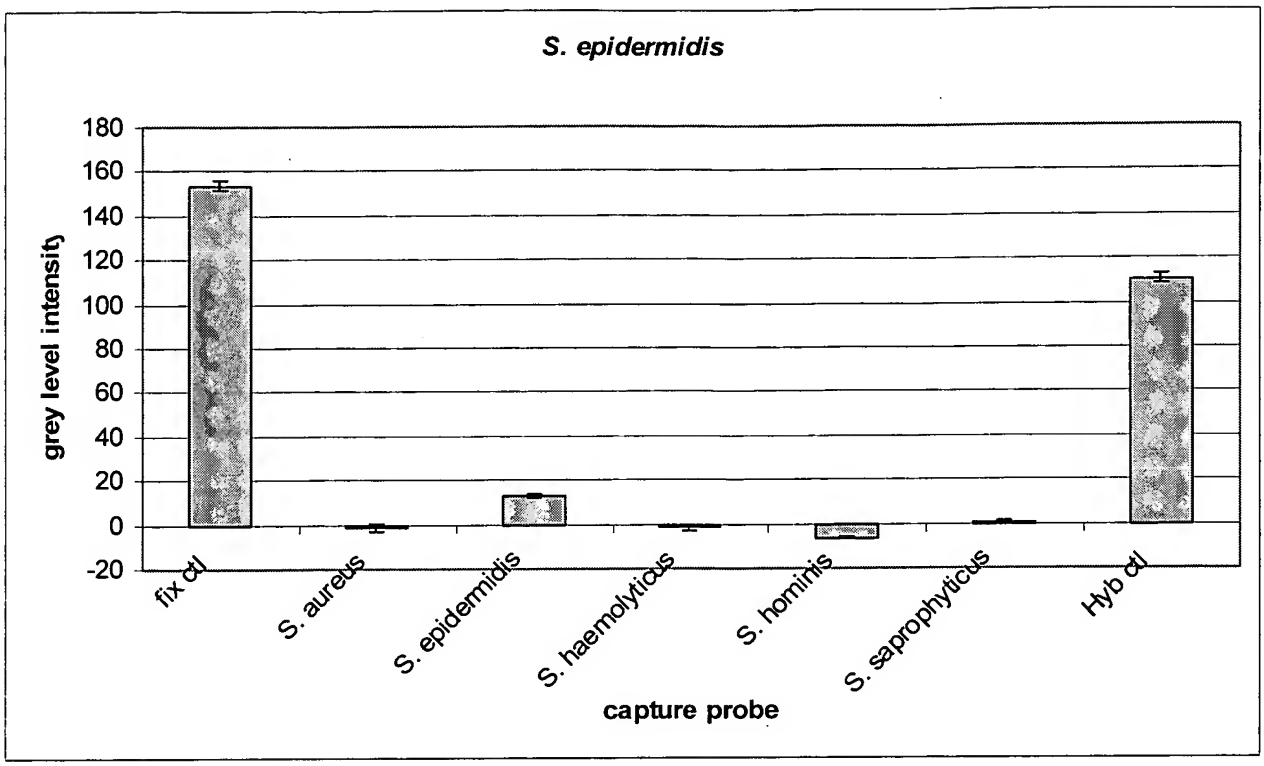

Exhibit 2: Detection of *S. epidermidis* after PCR and hybridization on DNA microarray having short specific capture probes for different Staphylococcus species according to the prior art (protocol of example 2 of the Specification). Fix.ctl is the fixation control for the probes. Hyb.ctl is the hybridization control.

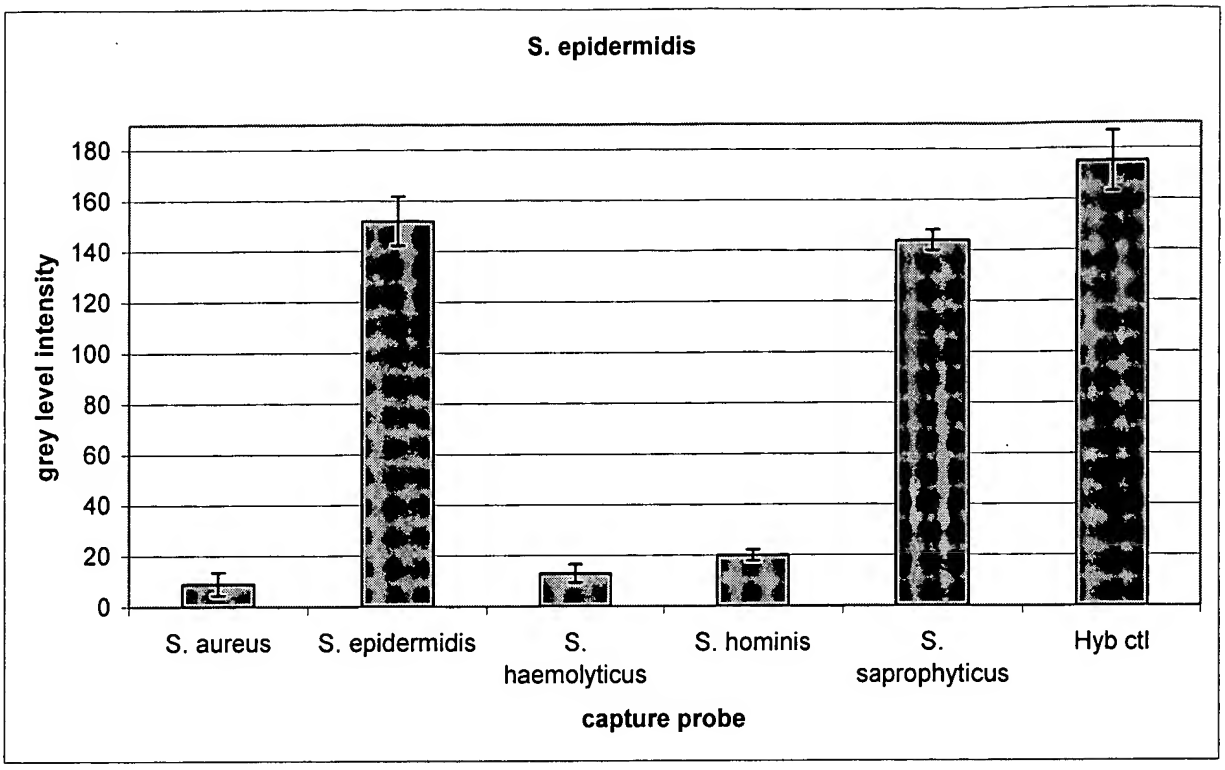

Exhibit 3: Detection of *S. epidermidis* after PCR and hybridization on DNA microarray having long specific capture probes (protocol of example 1 of the Specification). Hyb.ctl is the hybridization control.

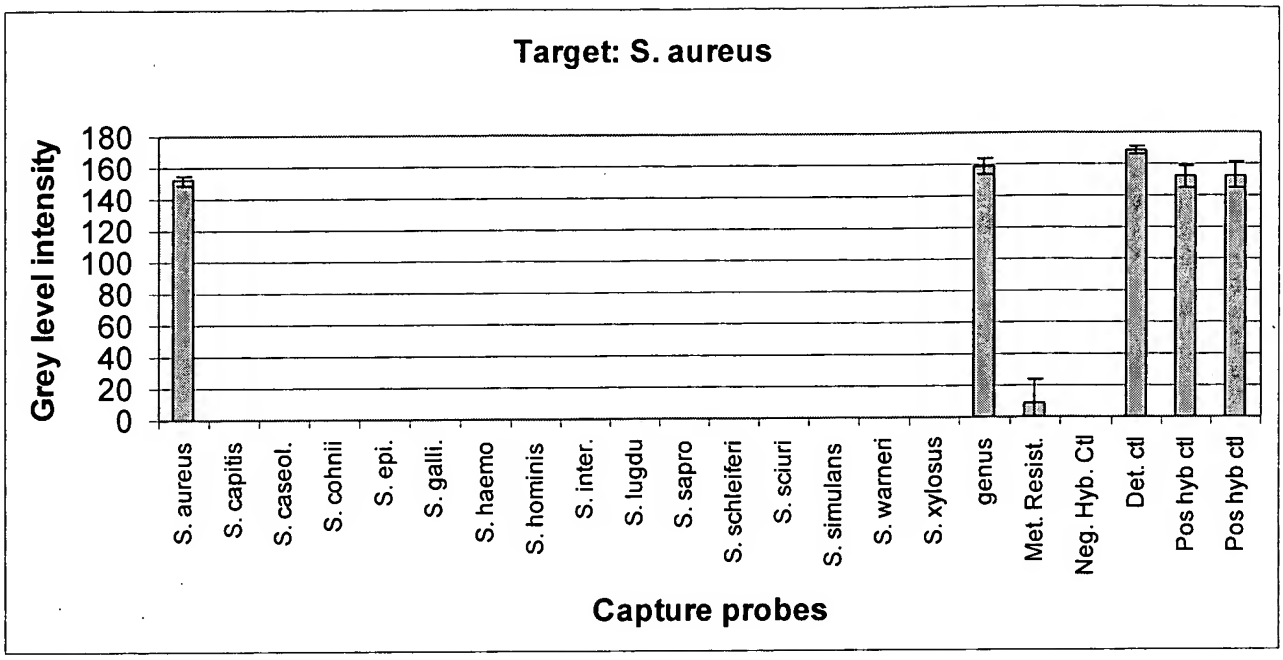

Exhibit 4: Detection of one bacteria species (*S. aureus*) after PCR using a consensus primer pair and hybridization on DNA microarray according to the invention on specific capture probes for different Staphylococcus species present or on a consensus probe for the Staphylococcus genus (protocol of examples 4 and 7 of the Specification). Det.ctl is the detection control. Poshyb ctl are the positive hybridization controls.

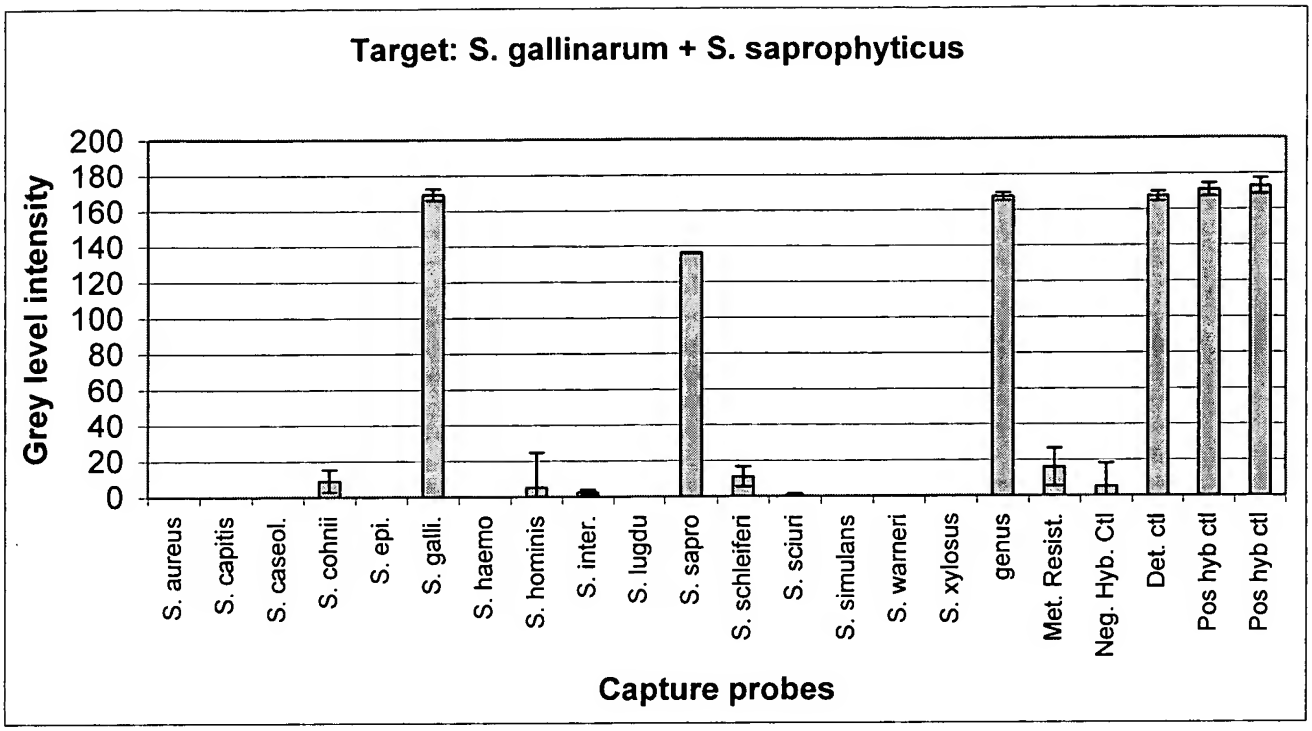

Exhibit 5: Simultaneous detection of two bacteria species (*S. gallinarum* and *S. saprophyticus*) after PCR using a unique consensus primer pair and on the microarray on specific capture probes for different Staphylococcus species present according to the invention or on a consensus probe for the Staphylococcus genus (protocol of examples 4 and 7 of the Specification). Det.ctl is the detection control. Poshyb.ctl are the positive hybridization controls

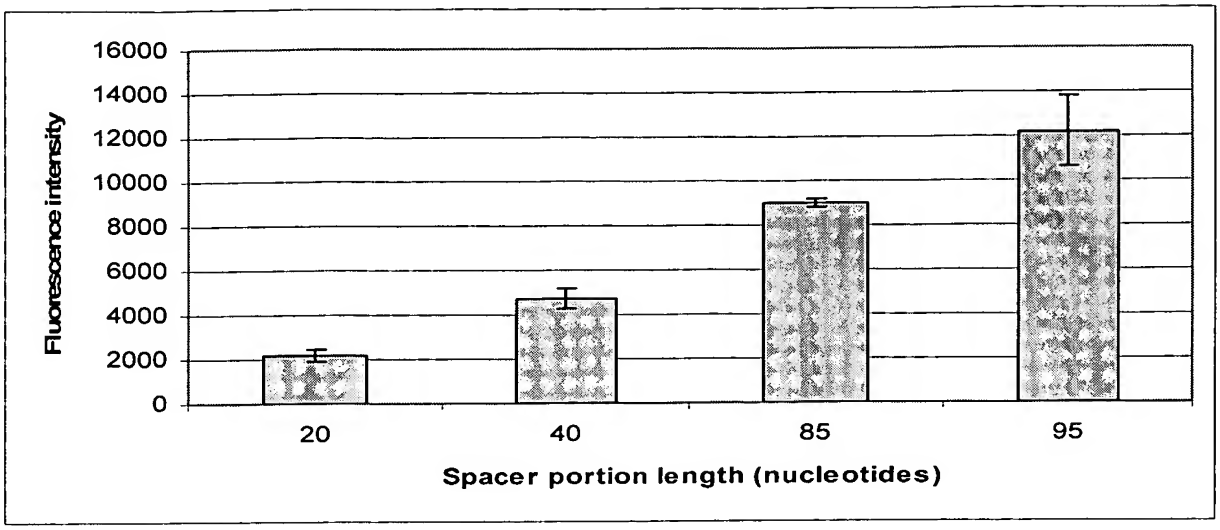

Exhibit 6: Effect of the spacer length on the hybridization yield (fluorescence intensity). The spacer length ranges from 20 to 95 bases with a constant sequence of 27 bases which is specific of the target molecule. The amplicons were obtained from amplification of *S. epidermidis FemA* gene by PCR

CV

Name: Prof. José Remacle

Institute: URBC, University of Namur, 61 Rue de Bruxelles, 5000 Namur, Belgium
Jose.remacle@fundp.ac.be. Director of the laboratory

Eppendorf Array Technologies, sa 20 Rue du Séminaire , 5000, Namur, Belgium
Remacle.j@eppendorf.be. Director of the R-D

Education and profession:

1970	PhD Department of Science, University of Louvain, Belgium
1973	Postdoc, Rockefeller University, NY, USA
1974	Postdoc, UCSD, San Diego, USA
1974	Professor University of Namur, Belgium
1999	Founder of the spin off company "Advanced Array Technology"
2002	Director R-D Eppendorf Array Technologies (EAT)

Research Interests: Development of new DNA and protein biochips platform for genomic and for gene expression. Use of protein chips in complementation to the proteomic analysis by 2-D gel and mass spectra analysis. Applications for research as well as for diagnostic applications such as cancer prognostic and bacterial identification. Numerous collaborations with many research laboratories. Development and production of 8 biochips of gene expression analysis and genomic chips for the detection of bacteria.

Professional Experience:
Joined the University of Namur in 1974 and starting the laboratory for biochemistry and cell biology. Head of the laboratory since 1974.
Starting the research unit in cell biology (URBC) in 1997. The URBC is now composed of 65 researchers and technicians.
Member of many national and international committees, EEC expert in biotechnology programs.
R-D Director of Eppendorf Array Technologies, S.A.

Publications

The author's scientific output consists of 351 research papers in peer-reviewed international journals and inventor of 47 filed patents

5 Publications 5 last years

1. I. Alexandre, S. Hamels, S. Dufour, J. Collet, N. Zammatteo, F. De Longueville, J.-L. Gala and J. Remacle, Colorimetric Silver Detection of DNA Microarrays, Anal. Biochem., 295, 1-8, 2001 (Impact fact. 0.332).
2. I. Alexandre, Y. Houbion, J. Collet, S. Hamels, J. Demarteau, J.-L. Gala and J. Remacle, Compact Disc with both numeric and genomic information as DNA microarray platform, Biotechniques, 33, 435-439, 2002 (Impact fact. 1.946).
3. T. Arnould, R. Thibaut-Vercruyssen, N. Bouaziz, M. Dieu, J. Remacle and C. Michiels, PGF2a, Prostanoid Released by Endothelial Cells Activated by Hypoxia, Is a Chemoattractant Candidate for Neutrophil Recruitment, American Journal of Pathology, 159, 345-357, 2001 (Impact fact. 7.246).
4. T. Arnould, S. Vankoningsloo, P. Renard, A. Houbion, N. Ninane, C. Demazy, J. Remacle and M. Raes, CREB activation induced by mitochondrial dysfunction is a new signaling that impairs cell proliferation, EMBO J, 21, 53-63, 2002 (Impact fact. 13.255).
5. N. Berna, T. Arnould, J. Remacle and C. Michiels, Hypoxia-induced increase in intracellular calcium concentration in endothelial cells: role f the Na+-glucose cotransporter, J Cell Biochem, 84, 115-131, 2002 (Impact fact. 3.471).
6. G. Bestel-Corre, E. Dumas-Gaudot, V. Poinsot, M. Dieu, J.-F. Dierick, D. Van Tuinen, J. Remacle, V. Gianinnazzi-Pearson and S. Gianinazzi, Proteome analysis and identification of symbiosis-related proteins from Medicago truncatula, Electrophoresis, 23, 122-137, 2002 (Impact fact. 2.467).
7. F. Bieswal, M. T. Ahn, B. Reusens, P. Holvoet, M. Raes, W. D. Rees and C. Remacle, The importance of catch-up growth after early malnutrition for the programming of obesity, Obesity Research, submitted (Impact fact. 3.409).
8. N. Bouaziz, M. Redon, L. Quéré, J. Remacle and C. Michiels, Mitochondrial respiratory chain as a new target for anti-ischemic molecules, Eur. J. Pharm., 441, 35-45, 2002 (Impact fact. 1.96).
9. F. Chainiaux, J. P. Magalhaes, F. Eliaers, J. Remacle and O. Toussaint, UVB-induced premature senescence of human diploid skin fibroblasts, Int J Biochem Cell Biol, 34, 1331-9, 2002 (Impact fact. 1.124).
10. F. Chainiaux, J. Remacle and O. Toussaint, Exposure of skin human diploid fibroblasts to repeated subcytotoxic doses of UVB induces the overexpression of the TGF-B1 mRNA, Ann. N.Y. Acad. Sci., 973, 44-48, 2002 (Impact fact. 1030).
11. F. De Longueville, F. A. Atienzar, L. Marcq, S. Dufrane, S. Evrard, L. Wouters, F. Leroux, V. Bertholet, B. Gerin, R. Whomsley, T. Arnould, J. Remacle and M. Canning, Use a low-density microarray for studying gene expression patterns induced by hepatotoxicants on primary cultures of rat hepatocytes., Toxicological Sciences, 75, 378-392, 2003 (Impact fact. 2.734).
12. F. De Longueville, D. Surry, G. Meneses-Lorente, V. Bertholet, V. Talbot, S. Evrard, N. Chandelier, A. Pike, P. Worboys, J. P. Rasson, B. Le Bourdellès and J. Remacle, Gene expression profiling of drug metabolism and toxicology markers using a low-density DNA microarray, Biochemical Pharmacology, 64, 137-149, 2002 (Impact fact. 2.612).
13. J. P. de Magalhaes, F. Chainiaux, F. De Longueville, V. Mainfroid, V. Migeot, L. Marcq, J. Remacle, M. Salmon and O. Toussaint, Gene expression and regulation in H2O2-induced premature senescence of human fibroblasts expressing or not telomerase, Experimental gerontology, 39, 1379-1389, 2004 (Impact fact. 2,493).
14. J. P. de Magalhaes, F. Chainiaux, J. Remacle and O. Toussaint, Stress-induced premature senescence in BJ and hTERT-BJ1 human foreskin fibroblasts, FEBS Lett, 523, 157-62., 2002 (Impact fact. 3.750).

15. F. Debacq-Chainiaux, C. Borlon, T. Pascal, V. Royer, F. Eliaers, N. Ninane, G. Carrard, B. Friguet, F. De Longueville, S. Boffe, J. Remacle and O. Toussaint, Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-β1 signaling pathway, Journal of Cell Science, 118, 743-758, 2005 (Impact fact.
16. J.-F. Dierick, M. Dieu, J. Remacle, M. Raes, P. Roepstorff and O. Toussaint, Proteomics in experimental gerontology, Experimental gerontology, 37, 721-734, 2002 (Impact fact. 2.493).
17. J.-F. Dierick, T. Pascal, F. Chainaux, F. Eliaers, J. Remacle, P. Mose Larsen, P. Roepstorff and O. Toussaint, Transcriptome and proteome analysis in human senescent fibroblasts and fibroblasts undergoing premature senescence induced by repeated sublethal stresses, in "Molecular and Cellular Gerontology", (Eds), Annals of the New York Academy of Sciences, 908, 302-305, 2000.
18. J. F. Dierick, F. Eliaers, J. Remacle, M. Raes, S. J. Fey, P. M. Larsen and O. Toussaint, Stress-induced premature senescence and replicative senescence are different phenotypes, proteomic evidence, Biochem Pharmacol, 64, 1011-7, 2002 (Impact fact. 2.263).
19. J. F. Dierick, T. Pascal, F. Chainiaux, F. Eliaers, J. Remacle, M. Larsen, Roepstorff and O. Toussaint, Transcriptome analysis and proteome analysis in human sensescent fibroblasts and fibroblasts undergoing premature senescence induced by repeated sublethal stresses, Ann. NY Acad. Sci., 908, 302-305, 2000 (Impact fact. 1.030).
20. J. F. Dierick, F. Wenders, F. Chainiaux, J. Remacle, F. A. B. and O. Toussaint, Retrovirally mediated overexpression of peroxiredoxin VI increases the survival of WI-38 human diploid fibroblasts exposed to cytotoxic doses of tert-butylhydroperoxide and UVB, Biogerontology, 4, 125-131, 2003 (Impact fact. 1.938).
21. P. Dumont, L. Balbeur, J. Remacle and O. Toussaint, Appearance of biomarkers of in vitro ageing after successive stimulations of WI-38 fibroblasts with IL-1a: senescence associated b-galactosidase activity and morphotype transition, J. Anat., 197, 529-537, 2000 (Impact fact. 1.384).
22. P. Dumont, M. Burton, Chen, C. Frippiat, T. Pascal, J. F. Dierick, J. Remacle and O. Toussaint, Human diploid fibroblasts display a decreased level of c-fos mRNA at 72 hrs after exposure to sublethal H2O2 stress, Ann. NY Acad. Sci., 908, 306-311, 2000 (Impact fact. 1.030).
23. P. Dumont, M. Burton, Q. M. Chen, E. S. Gonos, C. Frippiat, J. B. Mazarati, F. Eliaers, J. Remacle and O. Toussaint, Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast, Free Rad. Biology & Medicine, 28, 361-373, 2000 (Impact fact. 3.783).
24. P. Dumont, F. Chainiaux, J. F. Dierick, J. Remacle and O. Toussaint, Vieillissement cellulaire et sénescence réplicative, in "Gérontologie préventive. Eléments de prévention du vieillissement pathologique", C. Trivalle (Eds), Masson Editions, 25-34, 2002.
25. P. Dumont, F. Chainiaux, F. Eliaers, C. Petropoulou, J. Remacle, C. Koch-Brandt, E. S. Gonos and O. Toussaint, Overexpression of apolipoprotein J in human fibroblasts protects against cytotoxicity and premature senescence induced by ethanol and tert-butylhydroperoxide, Cell Stress Chaperones, 7, 23-35, 2002 (Impact fact.
26. P. Dumont, V. Royer, C. Frippiat, T. Pascal, J. Remacle and O. Toussaint, Radicaux libres et antioxydants dans le vieillissement, in "Gérontologie préventive. Eléments de prévention du vieillissement pathologique", C. Trivalle (Eds), Masson Editions, 35-49, 2002.
27. I. Ernest, I. Alexandre, N. Zammatteo, M. Herman, A. Houbion, F. De Leener, K. Fransen, G. van der Groen and J. Remacle, Quantitative assay for group M (subtype A-H) and group O HIV-1 RNA detection in plasma, J. Virological Methods, 93, 1-14, 2001 (Impact fact. 1.557).
28. B. Foultier, L. Moreno-Hagelsieb, D. Flandre and J. Remacle, Comparison of DNA detection methods using nanoparticles and silver enhancement, IEE Proc. - Nanotbiotechnol., 152, 3-12, 2005 (Impact fact.

29. C. Frippiat, Q. M. Chen, J. Remacle and O. Toussaint, Cell cycle regulation in H2O2-induced premature senescence of human diploid fibroblasts and regulatory control exerted by the papilloma virus E6 and E7 proteins, Exp. Gerontol., 35, 733-745, 2000 (Impact fact. 1.629).
30. C. Frippiat, Q. M. Chen, S. Zdanov, J. P. Magalhaes, J. Remacle and O. Toussaint, Subcytotoxic H2O2 stress triggers a release of transforming growth factor-b1, which induces biomarkers of cellular senescence of human diploid fibroblasts, J. Biol. Chem., 276, 2531-2537, 2001 (Impact fact. 7.452).
31. C. Frippiat, J. Dewelle, J. Remacle and O. Toussaint, Signal transduction in H2O2-induced senescence-like phenotype in human diploid fibroblasts, Free Rad. Biol. & Medicine, 33, 1334-1346, 2002 (Impact fact. 3.783).
32. C. Frippiat, J.-F. Dierick, J. Remacle and O. Toussaint, Consideration of heteroduplexes and homoduplexes for the quantification by competitive PCR of human mitochondrial DNA deletions with ageing of tissues and cells, Biogerontology, 1, 279-283, 2000 (Impact fact.
33. C. Frippiat, J. Remacle and O. Toussaint, Down-regulation and decreased activity of cyclin-dependent kinase 2 in H2O2-induced premature senescence, The International Journal of Biochemstry & Cell Biology, 1361, 1-9, 2002 (Impact fact. 1124).
34. J.-P. Gillet, T. Efferth, S. D., J. Hamels, F. De Longueville, V. Bertholet and J. Remacle, Microarray-based detection of Multidrug Resistance in Human Tumor Cells by Expression Profiling of ATP-binding Cassette Transporter Genes, Cancer Research, 34, 8987-8993, 2004 (Impact fact. 8,649).
35. S. Hamels, J.-L. Gala, S. Dufour, P. Vannuffel, N. Zammatteo and J. Remacle, Consensus PCR and microarray for diagnosis of the genus Staphylococcus, species, and Methicillin resistance, Biotechniques, 31, 1364-1372, 2001 (Impact fact. 1.946).
36. L. Hevesi, L. Jeanmart and J. Remacle, Method for obtaining a surface activation of a solid support for building biochips microarrays, brevet EU 00870184.9 filed in August 2000, 2000.
37. D. Janssens, E. Delaive, A. Houbion, F. Eliaers, J. Remacle and C. Michiels, Effect of venotropic drugs on the respiratory activity of isolated mitochondria and in endothelial cells, British Journal of Pharmacology, 130, 1513-1524, 2000 (Impact fact. 4.075).
38. D. Janssens, E. Delaive, J. Remacle and C. Michiels, Protection by bilobalide of the ischaemia-induced alterations of the mitochondrial respiratory activity, Fundam. Clin. Pharm., 14, 193-201, 2000 (Impact fact. 1.053).
39. M. Lacroix, N. Zammatteo, J. Remacle and G. Leclercq, A low-density DNA microarray for analysis of markers in breast cancer, The International Journal of Biological Markers, 17, 5-23, 2002 (Impact fact. 1.467).
40. F. Lebon, M. Ledecq, M. Dieu, C. Demazy, J. Remacle, R. Lapouyade, O. Kahn and F. Durant, Synthesis and structural analysis of the copper(II) complexes of N2-(2-pyridylmethyl)-2-pyridinecarboxamide, J. Inorg. Biochem, 86, 547-554, 2001 (Impact fact. 2.343).
41. J. Legault, C. Carrier, P. Petrov, P. Renard, J. Remacle and M. E. Mirault, Mitochondrial GPx1 decreases induced but not basal oxidative damage to mtDNA in T47D cells, Biochem. Biophys. Res. Commun., 272, 416-422, 2000 (Impact fact. 2.872).
42. G. Michel, E. Minet, I. Ernest, I. Roland, F. Durant, J. Remacle and C. Michiels, A model for the complex between the hypoxia-inducible factor-1 (HIF-1) and its consensus DNA sequence, J. Biomolecular Structure & Dynamics, 18, 2000 (Impact fact. 1.500).
43. G. Michel, E. Minet, D. Mottet, J. Remacle and C. Michiels, Site-directed mutagenesis studies of the hypoxia-inducible factor-1a DNA-binding domain, Biochim. Biophys. Acta, 1578, 73-83, 2002 (Impact fact. 2.430).
44. C. Michiels, T. Arnould and J. Remacle, Endothelial cell responses to hypoxia: initiation of a cascade of cellular interactions, Biochim. Biophys. Acta, 1497, 1-10, 2000 (Impact fact. 3.17).

45. C. Michiels, D. Mottet, E. Delaive, M. Dieu, J.-F. Dierick and J. Remacle, Cardiovascular genomics: new pathophysiological concepts, Proceedings of the 2001 European Science Foundation Workshop, at "Cardiovascular proteomics: effect of hypoxic conditions", P. A. Doevendans and S. Kääb (Eds), Maastricht, Kluwer Acadmic Publishers, 21-28, 2001.
46. C. Michiels, J. Remacle and N. Bouaziz, Endothelium and Venotropic Drugs in Chronic Venous Insufficiency: review, Phlebology, 17, 145-150, 2002.
47. E. Minet, T. Arnould, G. Michel, I. Roland, D. Mottet, M. Raes, J. Remacle and C. Michiels, ERK activation upon hypoxia: involvement in HIF-1 activation, FEBS Let., 468, 53-58, 2000 (Impact fact. 3.750).
48. E. Minet, G. Michel, J. Remacle and C. Michiels, Role of HIF-1 as a transcription factor involved in embryonic development, cancer progression and apoptosis (Review), Int. J. Molecular Medicine, 5, 253-259, 2000 (Impact fact. 1.689).
49. R. Pampin, B. Foultier, J.-P. Raskin, J. Remacle and D. Flandre, DNA analytical system-on-a-chip, in "Encyclopedia of sensors", (Eds), 2005.
50. R. Pampin, B. Foultier, J.-P. Raskin, J. Remacle and D. Flandre, DNA Analytical CMOS Systems-On-a-Chip, in "Encyclopedia of Sensors", E. C. D. a. M. V. P. C.A. Grimes (Eds), 1-18, 2006.
51. T. Pascal, F. Debacq-Chainiaux, A. Chrétien, C. Bastin, A.-F. Dabée, V. Bertholet, J. Remacle and O. Toussaint, Comparison of replicative senescence and stress-)induced premature senescence combining differential display and low-density DNA arrays, FEBS Lett, 579, 3651-3659, 2005 (Impact fact. 3,609).
52. J.-P. Piret, T. Arnould, B. Fuks, P. Chatelain, J. Remacle and C. Michiels, Caspase activation precedes PTP opening in TNF-a-induced apoptosis in L929 cells, Mitochondrion, 3, 261-278, 2004 (Impact fact. 1.65).
53. J.-P. Piret, T. Arnould, B. Fuks, P. Chatelain, J. Remacle and C. Michiels, Mitochondria permeability transition-dependent tert-butyl hydroperoxide-induced apoptosis in hepatoma HepG2 cells, Biochem Pharmacol, 67, 611-620, 2004 (Impact fact. 3.34).
54. J. Remacle, Des biopuces sur le marché, Athéna, 151, 453-456, 2000 (Impact fact.
55. J. Remacle, AAT S.A. et la génomique, La Revue Générale, 02, 83-87, 2001 (Impact fact.
56. J. Remacle, I. Alexandre, I. Ernest and N. Zammatteo, Method and kit for diagnosing and/or quantifying by sandwich hybridization of nucleic acid sequences on solid support, Brevet PCT/BE97/00102 filed in 1997, 2000.
57. J. Remacle, M. Art, L. Lockman and N. Zammatteo, Reverse detection for identification and/or quantification of nucleotide target sequences on biochips, brevet EU 00870127.8 filed in June 2000, 2000.
58. J. Remacle, S. Hamels, N. Zammatteo, L. Lockman, S. Dufour, I. Alexandre and F. De Longueville, Method and kit for detection and/or quantification of homologous nucleotide sequences on arrays, Brevet EU 00870055.1 filed in March 2000, 2000.
59. J. Remacle, P. Renard and M. Art, Method and kit for the screening and/or the quantification of transcriptional factors, Brevet EU 00870057.7 filed in March 2000, 2000.
60. P. Renard, E. Delaive, M. Van Steenbrugge, J. Remacle and M. Raes, Is the effect of interleukin-1 on glutathione oxidation in cultured human fibroblasts involved in NF-kB activation? Antioxidant and Redox Signalling, 2, 329-40, 2001 (Impact fact.
61. P. Renard, I. Ernest, A. Houbion, M. Art, H. Le Calvez, M. Raes and J. Remacle, Development of a sensitive multi-well colorimetric assay for active NF-kB, Nucleic Acids Res., 29, 1-5, 2001 (Impact fact. 4.488).
62. I. Roland, E. Minet, I. Ernest, T. Pascal, G. Michel, J. Remacle and C. Michiels, Identification of hypoxia-responsive messengers expressed in human microvascular endothelial cells using differential display RT-PCR, Eur. J. Biochem., 267, 3567-3574, 2000 (Impact fact. 3.275).

63. M. Salmon, J. Dedessus Le Moutier, F. Wenders, S. Chiarizia, F. Eliaers, J. Remacle, V. Royer, T. Pascal and O. Toussaint, Role of the PLA2-independent peroxiredoxin VI activity in the survival of immortalized fibroblasts exposed to cytotoxic oxidative stress, FEBS Lett, 557, 26-32, 2004 (Impact fact. 3.644).
64. O. Toussaint, P. Dumont, J.-F. Dierick, T. Pascal, C. Frippiat, F. Chainiaux, J.-P. Magalhaes, F. Eliaers and J. Remacle, Stress-induced premature senescence as alternative toxicological method for testing the long-term effects of molecules under development in the industry, Biogerontology, 1, 177-181, 2000 (Impact fact. 1.938).
65. O. Toussaint, P. Dumont, J. F. Dierick, T. Pascal, C. Frippiat, F. Chainiaux, F. Sluse, F. Eliaers and J. Remacle, Stress-induced premature senescence. Essence of life, evolution, stress and aging, Ann. NY Acad. Sci., 908, 85-98, 2000 (Impact fact. 1.030).
66. O. Toussaint, P. Dumont, J. F. Dierick, T. Pascal, S. Mazarati, A. Barez, F. Eliaers and J. Remacle, Réponse à long terme aux stress subléthaux = vieillissement cellulaire accéléré? in "Proceedings 21th Winter Meeting Gerontologie & Geriatrie", Baeyens (Eds), Garant, 23-38, 2002.
67. O. Toussaint, P. Dumont, J. Remacle, J. F. Dierick, T. Pascal, C. Frippiat, J. P. Magalhaes, S. Zdanov and F. Chainiaux, Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? How stress, cellular behaviors, growth kinetics and cell heterogeneity interact in senescence, Scientific World J., 2, 230-247, 2002 (Impact fact.
68. O. Toussaint, P. Lemaire, P. Dumont, J.-F. Dierick, T. Pascal, C. Frippiat, F. Chainaux, J. P. Magalhaes, F. Eliaers, A.-F. Dabee and J. Remacle, Nutrition, vieillissement et modèles in vitro permettant de tester les effets à long terme potentiels d'additifs alimentaires en développement dans l'industrie agro-alimentaire, in "L'impact de la nutrition sur la santé", D. Provoost (Eds), Garant. Louvain, 79-86, 2000.
69. O. Toussaint, J. Remacle, B. F. C. Clark, E. S. Gonos, C. Franceschi and T. B. L. Kirkwood, Molecular, Cellular and Tissue Gerontology, Bioessays, 22, 954-956, 2000 (Impact fact. 6.227).
70. O. Toussaint, J. Remacle, J. F. Dierick, T. Pascal, C. Frippiat, V. Royer and F. Chainiaux, Approach of evolutionary theories of ageing, stress, senescence-like phenotypes, calorie restriction and hormesis from the view point of far- from-equilibrium thermodynamics, Mech Ageing Dev, 123, 937-46, 2002 (Impact fact. 0.890).
71. O. Toussaint, J. Remacle, J. F. Dierick, T. Pascal, C. Frippiat, V. Royer, J. P. Magalhaes, S. Zdanov and F. Chainiaux, Stress-induced premature senescence: from biomarkers to likeliness of in vivo occurence, Biogerontology, 3, 13-17, 2002 (Impact fact. 1.938).
72. O. Toussaint, J. Remacle, J. F. Dierick, T. Pascal, C. Frippiat, S. Zdanov, J. P. Magalhaes, V. Royer and F. Chainiaux, From the Hayflick mosaic to the mosaics of ageing. Role of stress- induced premature senescence in human ageing, Int J Biochem Cell Biol, 34, 1415-29, 2002 (Impact fact. 1.124).
73. O. Toussaint, V. Royer, M. Salmon and J. Remacle, Stress-induced premature senescence and tissue ageing, Biochem Pharmacol, 64, 1007-9, 2002 (Impact fact. 2.612).
74. S. Vankoningsloo, A. De Pauw, A. Houbion, S. Tejerina, C. Demazy, F. de Longueville, V. Bertholet, P. Renard, J. Remacle, P. Holvoet, M. Raes and T. Arnould, CREB activation induced by mitochondrial dysfunction triggers triglyceride accumulation in 3T3-L1 preadipocytes, J Cell Sci, 2006 (Impact fact. 7.25 - 0021-9533 (Print)
Journal article).
75. S. Warnon, N. Zammatteo, I. Alexandre, C. Hans and J. Remacle, Colorimetric detection of the tuberculosis complex using cycling probe technology and hybridization in microplates, Biotechniques, 28, 1152-1160, 2000 (Impact fact. 1.946).

76. N. Zammatteo, S. Hamels, F. De Longueville, I. Alexandre, J.-L. Gala, F. Brasseur and J. Remacle, New chips for molecular biology and diagnostics, in "Biotechnology", M. R. El-Gewely (Eds), Elsevier, 8, 85-101, 2002.
77. N. Zammatteo, L. Jeanmart, S. Hamels, S. Courtois, P. Louette, L. Hevesi and J. Remacle, Comparison between different strategies of covalent attachment of DNA to glass surfaces to build DNA microarrays, Anal. Biochem., 280, 143-150, 2000 (Impact fact. 2.047).
78. N. Zammatteo, L. Lockman, F. Brasseur, E. de Plaen, C. Lurquin, P.-E. Lobert, S. Hamels, T. Boon and J. Remacle, DNA microarray to monitor the expression of MAGE-A genes, Clinical Chemistry, 48, 25-34, 2002 (Impact fact. 3.422).